Microbial Challenge Testing of Single Liquid Cathode Feed Water Electrolysis Cells for the International Space Station (ISS) Oxygen Generator Assembly (OGA)

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The International Space Station (ISS) Oxygen Generator Assembly (OGA) operational performance may be adversely impacted by microbiological growth and biofilm formation over the electrolysis cell membranes. Biofilms could hinder the transport of water from the bulk fluid stream to the membranes and increase the cell concentration overpotential resulting in higher cell voltages and a shorter cell life. A microbial challenge test was performed on duplicate single liquid-cathode feed water electrolysis cells to evaluate operational performance with increasing levels of a mixture of five bacteria isolated from ISS and Space Shuttle potable water systems. Baseline performance of the single water electrolysis cells was determined for approximately one month with deionized water. Monthly performance was also determined following each inoculation of the feed tank with 100, 1000, 10,000 and 100,000 cells/ml of the mixed suspension of test bacteria. Water samples from the feed tank and recirculating water loops for each cell were periodically analyzed for enumeration and speciation of bacteria and total organic carbon. While initially a concern, this test program has demonstrated that the performance of the electrolysis cell is not adversely impacted by feed water containing the five species of bacteria tested at a concentration measured as high as 1,000,000 colony forming units (CFU)/ml. This paper presents the methodologies used in the conduct of this test program along with the performance test results at each level of bacteria concentration.

I. Introduction

THE Oxygen Generator Assembly on-board the International Space Station produces oxygen and hydrogen from the electrolysis of water. The source of water for the OGA is the potable water bus on the ISS. Originally specified to have a maximum microbial load of 1 CFU/ml, the requirement for potable water has been relaxed to 50 CFU/ml. Since all ground test programs of the development and flight hardware were conducted at the original,

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more stringent levels, there was no data demonstrating that the hardware could operate at the higher levels now permitted by the specification. A test program was developed whereby the performance of water electrolysis cells was evaluated with an increasing challenge of microbial content in the feed water.

Two liquid-cathode feed single cell assemblies were placed on test in a specially designed test system in the Electrochemical Engineering Laboratory at Hamilton Sundstrand in Windsor Locks, CT (HSWL) to evaluate their performance when operating from feed water containing different concentration levels of microbial species. The specific goals for the test program were:

- Demonstrate that the normal cell degradation rate is not accelerated by nominally higher than design specification microbial loads in the water feeding the OGA.
- Identify an approximate upper limit for operation of the cells without adverse effect to the cell degradation rate.
- Determine if higher microbial levels that could occur during a microbial upset of the potable water bus would necessitate the shutdown of the OGA to protect the integrity of the water electrolysis cell stack.

Testing was conducted in essentially two phases. In the first phase, the single cells were operated with deionized water to establish a baseline for cell performance. During the second phase, the feed tank was inoculated with microbial species that are present or expected to be present in the ISS potable water bus.

II. Test Plan

A detailed test plan for conducting the baseline and microbial test program defined specific test objectives including the operating parameters for the single cell assemblies and test system, the microbial species to be introduced during the inoculation, the sampling protocol and the analytical methods to be employed for measuring the microbial content of the water samples.

Baseline - The initial phase of the test program established a baseline for cell performance using facility deionized water as the feed source for the water electrolysis cells. There were no specific microorganisms introduced as a challenge in this phase of testing. The facility DI water was not sterilized, so this represented a native microbial challenge to the electrolysis cells.

Microbial Challenge Testing - After the cell performance baseline was established, the concentration level of microbial species in the feed tank was increased incrementally. The challenge microorganisms were *Burkholderia cepacia, Cupriavidus metallidurans, Methylobacterium fujisawaense, Caulobacter vibrioides, and Ralstonia pickettii*, all waterborne microorganisms isolated from various ISS water samples.

The various microbial challenge microorganisms were streaked, harvested, washed, and diluted to McFarland Turbidity Standard #1 (55.6% Transmission which is equivalent to an approximate cell density of 3.0E+08 colony forming units (CFU)/ml) and cell counts were performed with a Petroff Hausser Counting Chamber at the Boeing Laboratory in Huntsville, AL.[1] After preparing equivalent concentrations of the five species of challenge microorganisms, the organisms were diluted with sterile DI water and combined into a stock mixed suspension that was sent to HSWL for introduction into the water reservoir that fed the two liquid-cathode feed single cell assemblies. HSWL inoculated the feed tank containing ~ 10-liters of non-sterile DI water filtered through a 0.22µ filter with 1-ml of a combined stock solution with the intent to increase the microbial challenge to the cells incrementally. The target levels of cell density chosen for this test were 100 CFU/ml, 1000 CFU/ml, 10,000 CFU/ml, and 100,000 CFU/ml.

Specific design requirements and operational constraints for the test program included:

- Both cells operated cyclically and at a production rate consistent with a three-member crew - 53 minutes at 50% production (23 amps), 37 minutes at STANDBY operation (1 amp). Additionally the single cells operated at a constant, nominal inlet temperature of 27°C (80°F), which is within the normal operating range for the OGA recirculation water loop. Consistency in cell temperature is an important factor in obtaining pertinent cell voltage degradation rate data.
- The plumbing used in each setup was 316 stainless steel and Teflon® to be consistent with the flight hardware design. The volume of the two loops was minimized and was constructed to be as close to each other as possible.
- All translucent/transparent components were wrapped with aluminum foil or insulating foam to prevent ambient light from affecting microbial growth in the test system plumbing.
- The two single cell test loops operated from a common feed water tank so that they both received the same challenge. With both cells operating at the same current, they consumed equal amounts of feed water and

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were therefore exposed to the same microbial challenge. Line lengths to each of the two loops were the same.

- All components used in the construction of the test system were cleaned to be free of dirt/greases/lubricants, and then were flushed with filtered DI water. Subsequent water samples from the feed tank and two recirculation loops to determine the efficacy of the cleaning procedure were analyzed and confirmed to have a conductivity of less than 1 μmhos/cm and a total organic carbon (TOC) content of less than 500 ppb. There was no requirement to sterilize any component in the test system as the water electrolysis cell is not sterile and samples of existing water recirculation loops from operational cells indicated very low levels of microbial activity.
- The feed tank was constructed of glass with a Teflon drain valve and was maintained at approximately 40°F. A magnetic stirrer was used to prevent stagnation of water within the tank, and a stainless steel line with a plug valve was inserted through a silicone rubber stopper at the top of the tank for introduction of the inoculum to the feed tank water supply as well as aseptically sampling the tank contents. The feed tank was sized to supply a single batch of feed water for 30 days plus a contingency of an additional 15 days of operation as well as pre-test, post-test, and weekly sampling volumes.
- All vents in the test system employed 0.22μ filters to prevent airborne contaminants from entering the test fluid.
- The water recirculation rate was established at the nominal flow rate for a single cell within the OGA of 150 cm^3/min (20 lb_m/hour).
- A specially-designed sampling port was constructed whereby a slip stream of water flowed constantly through the sampling valve to ensure no biofilms were formed due to flow stagnation. A representation of the sampling port design and a photograph of the completed unit are included as Fig. 1 and 2, respectively.

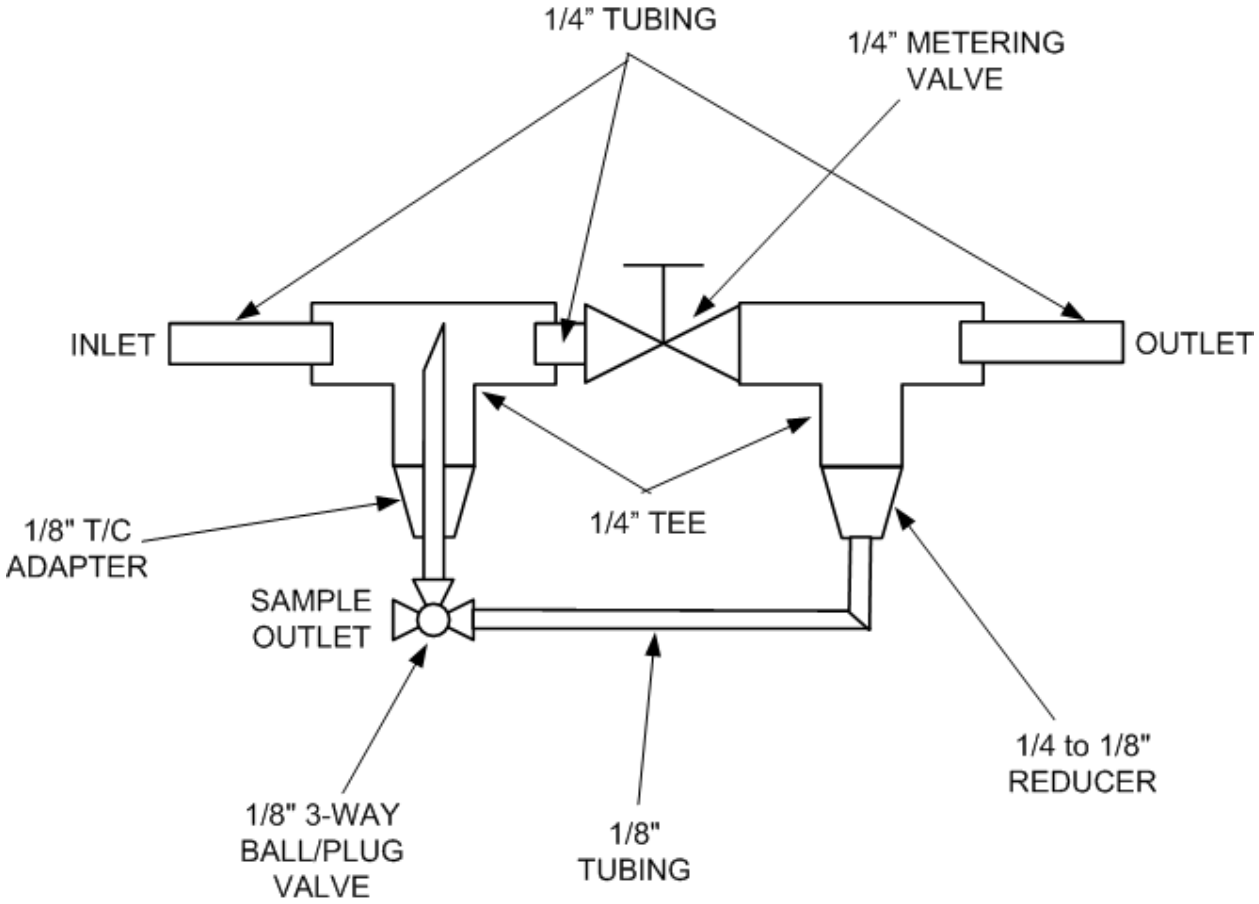

Figure 1. OGA Single Cell Microbial Challenge Sample Valve Drawing.

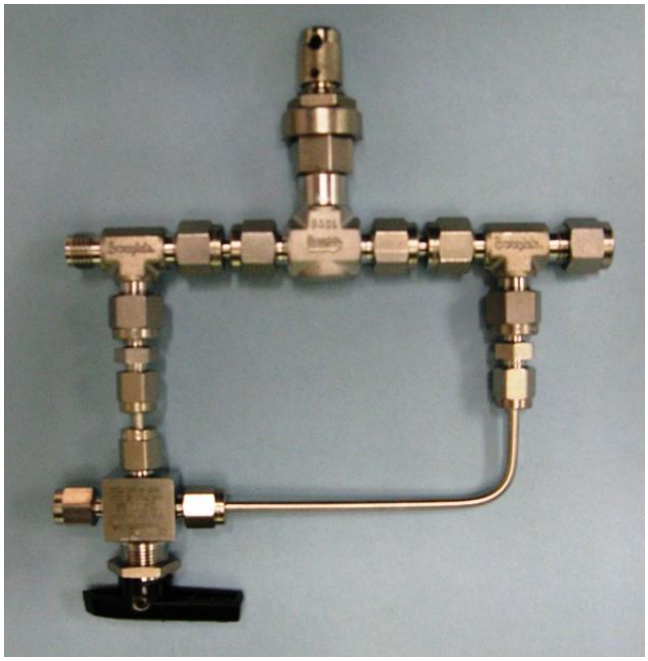

Figure 2. OGA Single Cell Microbial Challenge Sample Valve.

Periodically, 10-ml samples of inoculated challenge water were aseptically drawn from the test reservoir, and from each of the single cell assembly water recirculation loops and analyzed for bacterial counts as well as TOC content according to the schedule defined in Table 1. The samples for microbial analysis were refrigerated and subsequently sent overnight in a cooler to the Boeing Huntsville Laboratory for heterotrophic plate counts performed by membrane filtration on R2A incubated for 7 days at 28°C (82°F).[2] Representatives of each different colony morphology on a countable dilution were isolated into pure culture for identification. Identifications were performed using the Sherlock® Microbial Identification System fatty acid methyl ester gas chromatography, Biolog substrate utilization, and selected biochemical tests. The TOC analysis was conducted by HSWL using UV/persulfate oxidation coupled with non-dispersive infrared.

III. Test Item Description

The two test articles were liquid-cathode feed single cell assemblies that were initially built in August 2000 to support system-level trade studies for the ISS OGA as the operational characteristics of sub-ambient operating temperature and cyclic operation were examined. Assigned build designations 230OGA011-1 and 230OGA012-1, these single cells had operated for 49,876.7 hours and 47,721.5 hours respectively during that test program. The configuration of the two single cell assemblies is similar to the flight OGA cell stack from both a design and materials of construction perspective. A cross-sectional view of a single liquid-cathode feed cell is included as Fig. 3. A photograph of one of the single cell assemblies is included as Fig. 4. Each cell is compressed between (2) two-inch thick aluminum end plates that maintain proper seal load on the assembly, ensure adequate loading of the cell active area and provide a thermal sink for the cell to maintain near-isothermal operation.

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Table 1. Microbial Challenge Test.

Test Description	Frequency	Location	Microbial	TOC
Baseline (DI water)	start of test	feed tank	150 ml	40 ml
	weekly	recirculation loop	5 ml	-
	weekly	feed tank	5 ml	-
	end of test	recirculation loop	5 ml	20 ml
	end of test	feed tank	150 ml	40 ml
100 CFU/ml	start of test	feed tank	150 ml	40 ml
	weekly	recirculation loop	5 ml	-
	weekly	feed tank	5 ml	-
	end of test	recirculation loop	5 ml	20 ml
	end of test	feed tank	150 ml	40 ml
1000 CFU/ml	start of test	feed tank	150 ml	40 ml
	weekly	recirculation loop	5 ml	-
	weekly	feed tank	5 ml	-
	end of test	recirculation loop	5 ml	20 ml
	end of test	feed tank	150 ml	40 ml
10,000 CFU/ml	start of test	feed tank	150 ml	40 ml
	weekly	recirculation loop	5 ml	-
	weekly	feed tank	5 ml	-
	end of test	recirculation loop	5 ml	20 ml
	end of test	feed tank	150 ml	40 ml

IV. Test System Description

As previously stated, the test system was designed to operate two single-cell assemblies from a single water source. The two recirculating water loops are identical in construction to ensure near equal volumes in each of the loops. A schematic of the test system is included as Fig. 5; a photograph of the system (prior to installation of thermal and light-blocking insulation) is provided as Fig. 6.

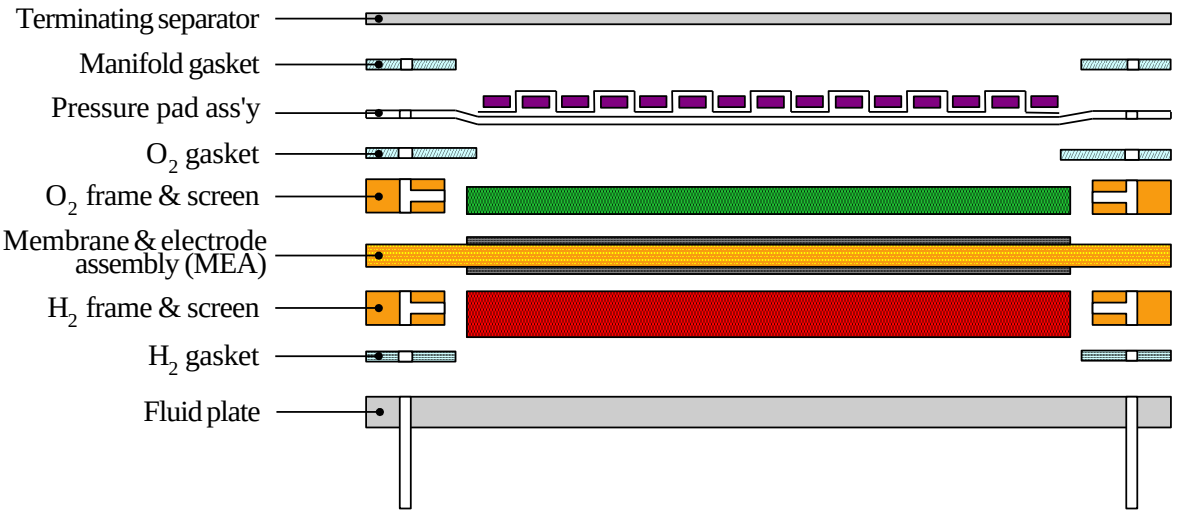

Figure 3. Liquid Cathode Feed Cell Schematic.

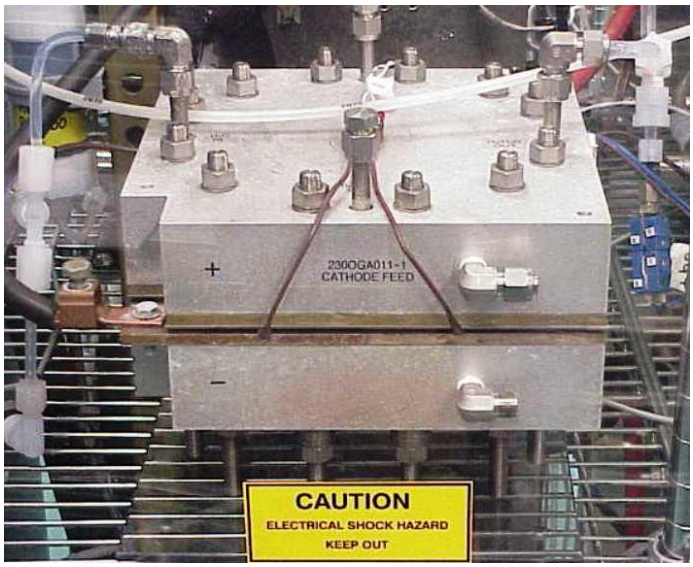

Figure 4. Liquid Cathode Feed Single Cell Assembly.

The source water for the two single-cell test articles is stored in a constantly-stirred 19-liter glass vessel (TK507). A chiller bath (CB600) circulates cold water through a perfluoroalkoxy (PFA) tubing coil placed inside the tank to maintain the source water at approximately 4°C (40°F) to control its microbial population. The tank is charged with deionized (DI) water through a 0.22μ polypropylene capsule filter (F501) from the laboratory DI water system. Tank water samples and the inoculums are withdrawn from or delivered through a plug valve at the top of the tank (V505). Water is delivered to either of the two fluid loops at the conclusion of each production cycle to satisfy the level sensors (L303) located in each of the hydrogen / water phase separators (PS302).

The water flow rate through each of the recirculating loops is maintained by a magnetically-driven gear pump (GP306) at 150 cm^3/min (20 lb_m/hr). A turbine flow sensor (FS312) monitors the flow rate and initiates an automatic shutdown of the test article and test system if the water flow rate drops below a preset value. A pressure sensor (P307) and pressure relief valve (RV308) ensure the recirculating loop pressure is maintained below 50 psi to guarantee the safety of the test article and test personnel. A needle valve (MV309) and three-way valve (DV310) provide a means for taking water samples from the recirculating water loop for microbial and TOC analysis. The tubing connection to the common port of the three-way valve is tapered and is inserted into the fluid stream. This feature, along with the setting of the needle valve, ensures a constant flow of water through the sampling leg to prevent any stagnant zones. A 1/8" tube stub approximately 1" long (not shown) was included at the remaining outlet port of the three-way valve to facilitate filling of the water sample bottle.

Water is fed to each of the single cells through their cathode, or hydrogen cavities. The hydrogen/water stream exiting the single cell is directed to a phase separator (PS302); the product hydrogen is vented to the laboratory ventilation system through a 0.22μ polypropylene capsule filter (F301) while the water is returned to the loop from the bottom of the separator column. The product oxygen is vented directly to the laboratory ventilation system through a condensate trap (PS200) without any filtration.

Thermal control of the recirculating water loop is accomplished by circulating warm water through the shell of a shell-and-tube heat exchanger (HX311) and the aluminum blocks that serve as endplates for the single cells. The single cells and water in the recirculating water loops are maintained at approximately 27°C (80°F) by a constant temperature bath (CB400). Thermocouples are located at the water inlet and outlet of the single cells as well as at the supply and return of the constant temperature bath to monitor the performance of the thermal control system.

The two single cells operate cyclically - 23 amps (50% production) for 53 minutes, followed by operation at 1 amp (standby) for 37 minutes. The current to the cell is incremented/decremented at a rate of approximately 1 amp/sec. The data acquisition and control system monitor and record the performance of the test system and the two single cells and initiate an automatic shutdown of the test if an out of tolerance condition occurs. A summary of the test system instrumentation and data recorded during the test program is included as Table 2.

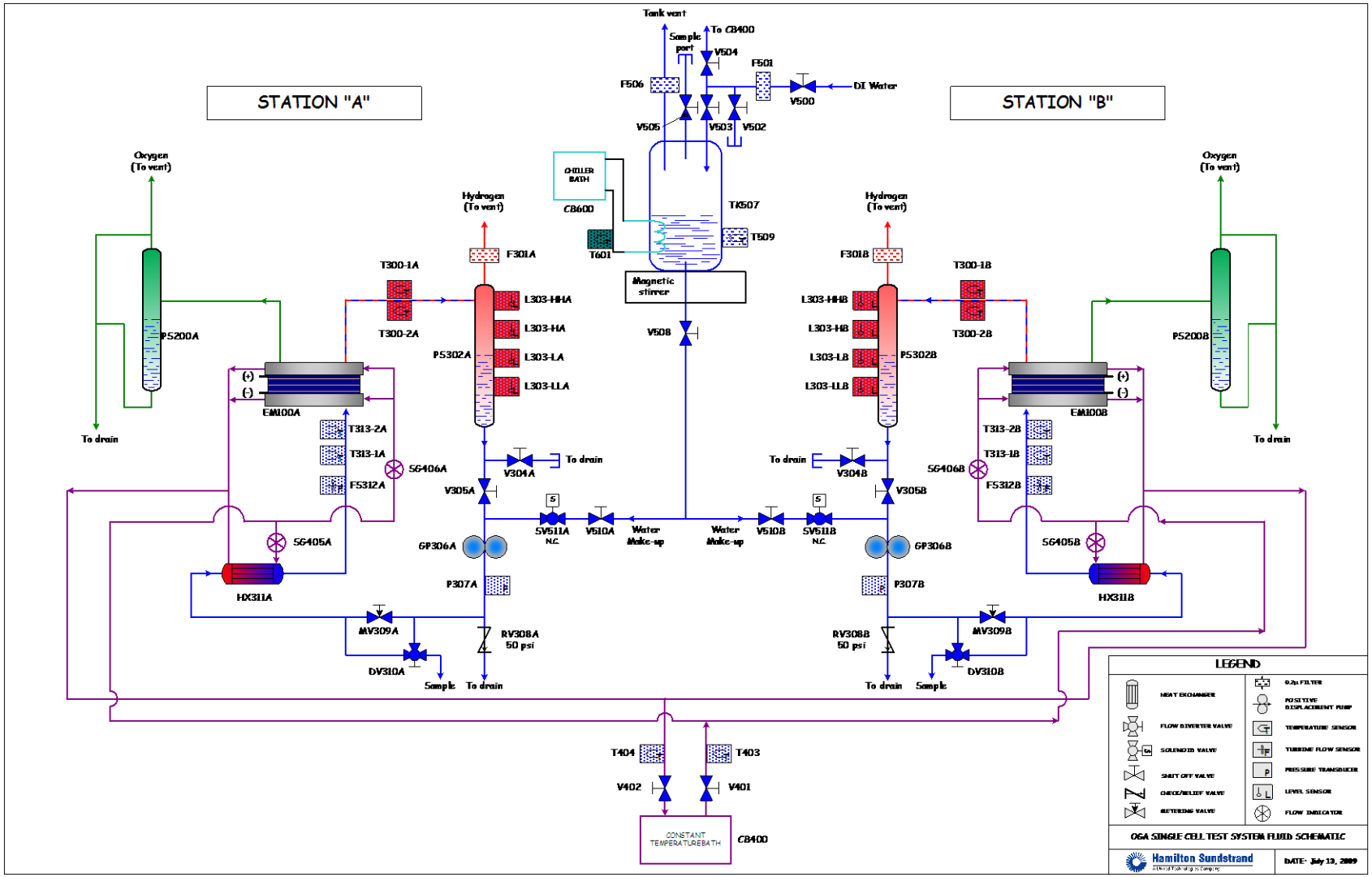

Figure 5. OGA Single Cell Microbial ChallengeTest System Fluid Schematic.

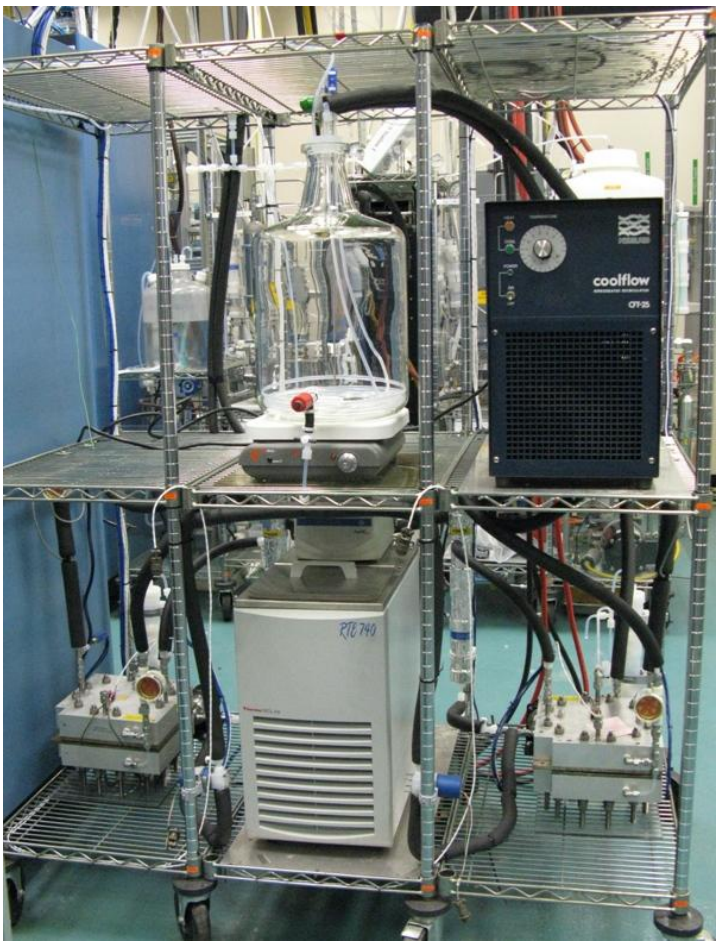

Figure 6. OGA Single Cell Microbial Challenge Test System.

Table 2. Single Cell Test Program – Instrumentation and Recorded Data.

Item Number	Description
EM100	Cell current & cell voltage
T300	Cell stack hydrogen/water outlet temperature
P307	Recirculating water loop pressure (at pump outlet)
FS312	Recirculating water loop flow rate
T313	Cell stack water inlet temperature
T403	Cell stack thermal control loop supply temperature
T404	Cell stack thermal control loop return temperature
T509	Source water temperature
T601	Source water supply tank chill water loop supply temperature

V. Baseline Test Results

The baseline test program using filtered DI water from the laboratory system was initiated on September 11, 2009. The initial water samples from the source tank and recirculating water loops were taken on September 14; subsequent water samples from the recirculating water loops were taken on a weekly basis. The baseline test program concluded on October 30 with the introduction of the first inoculums; total test time with DI water was 1156 hours or approximately 48 days.

Baseline microbiological counts in the feed tank were consistently greater than 1.0E+03 CFU/ml with the highest count at the end of the baseline testing of 7.75E+04 CFU/ml. The predominant species in the tank was *Duganella zoogloeoides*, a Gram negative soil bacterium that can convert nitrogen into ammonia. Also present at lower concentrations were other Gram negative species commonly found in soil and water including *Bradyrhizobium japonicum*, *Janthinobacterium lividum*, *Xanthobacter agilis*, and *Ralstonia pickettii*. Recirculation Loop A baseline counts ranged from 85 to 1725 CFU/ml and Recirculation Loop B counts ranged from 69 to 725 CFU/ml. Species present in both loops were *Ralstonia pickettii* and *Ralstonia solanacearum* and these Gram negative, non-fermentative bacteria are commonly found in water and soil. Reiculated Loop B also contained *Methylobacterium mesophilicum/radiotolerans*, another Gram negative soil bacterium.

The baseline performance curves of cell voltage versus operating time are presented in Fig. 7 through 10. The cell voltage data was parsed according to operating current to facilitate analysis of the data. The figure of merit for performance life testing of a cell is the degradation rate, typically measured in microvolts per hour. The degradation rates were calculated for both of the single cell assemblies at the 50% production level and at standby conditions using a simple linear regression analysis; the results are presented in Table 3. These degradation rates served as the basis for comparison for the subsequent source water challenges, beginning with the 100 CFU/ml challenge test program, which began on October 30. Cell degradation rates at or below these levels would provide indication that the performance of the water electrolysis cell was not adversely affected by the microbial population being evaluated at the time of test.

Thermal control of the test articles was very good, with only one minor excursion caused by a temporary fluctuation in the constant temperature bath (CB400) at approximately 247 hours. The water flow rate varied slightly depending on the operating current for the cell, with a slight drop noted as the current to the cell increased causing increased back pressure on the pump.

Water samples were taken periodically to evaluate the TOC and microbial population of the source tank and recirculating water loops according to Table 1. The analysis results are presented in Table 4. The TOC analysis at the conclusion of the baseline test program was not run to prevent significant dilution of the baseline microbial population and chemical parameters.

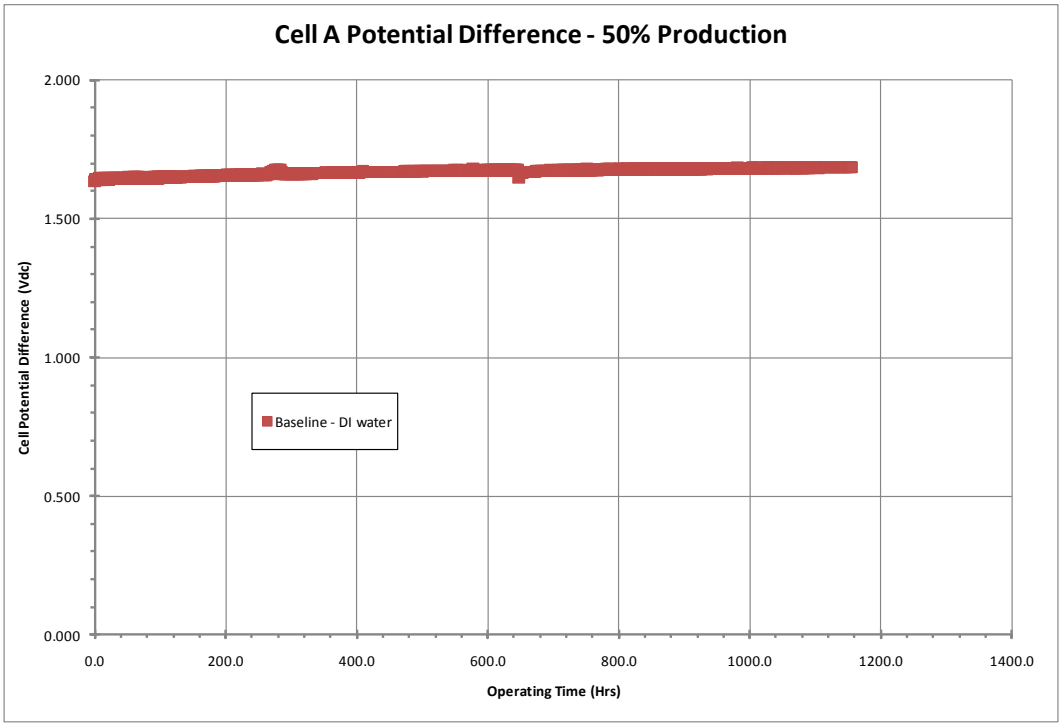

Figure 7. Single Cell Performance - Station A - 50% Production.

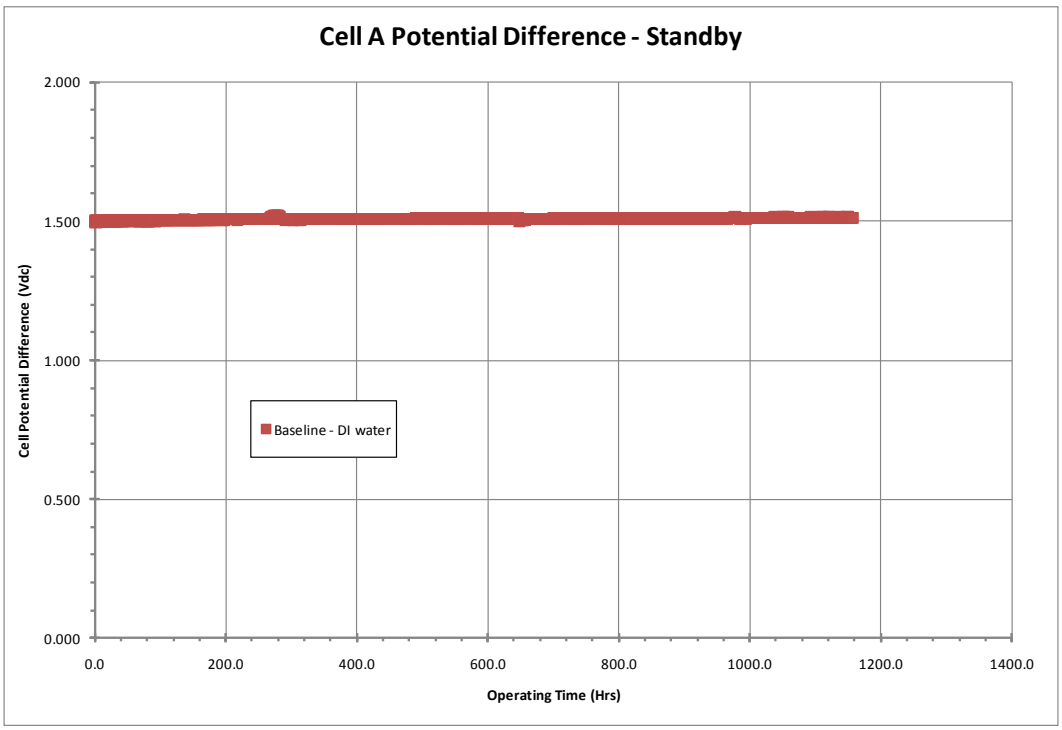

Figure 8. Single Cell Performance - Station A – Standby.

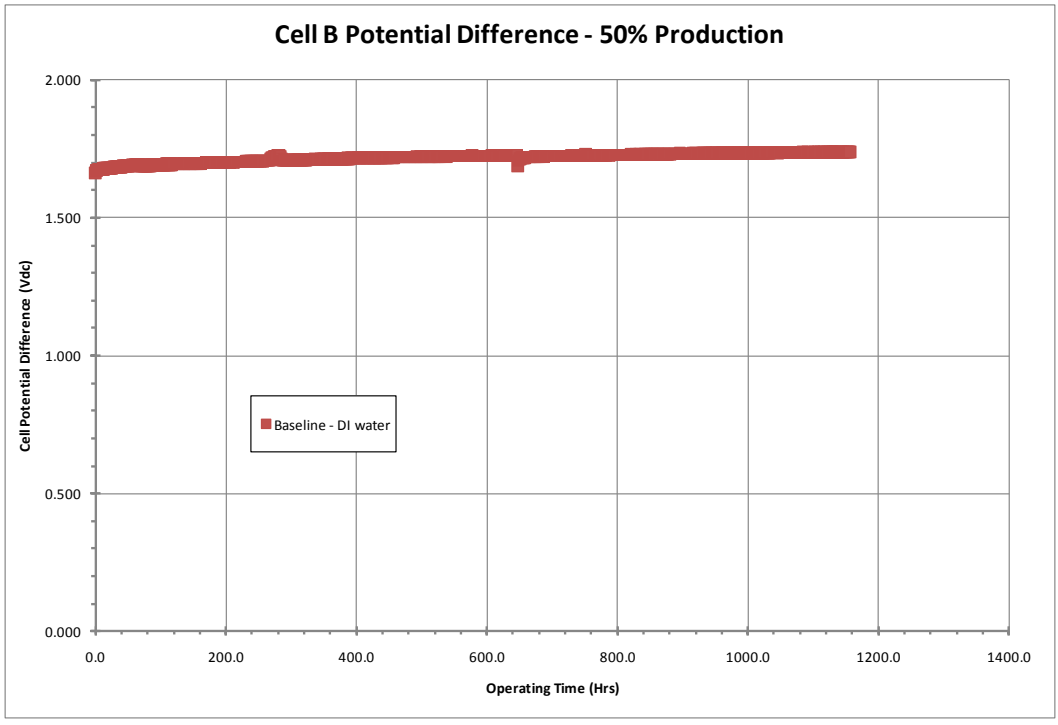

Figure 9. Single Cell Performance - Station B - 50% Production.

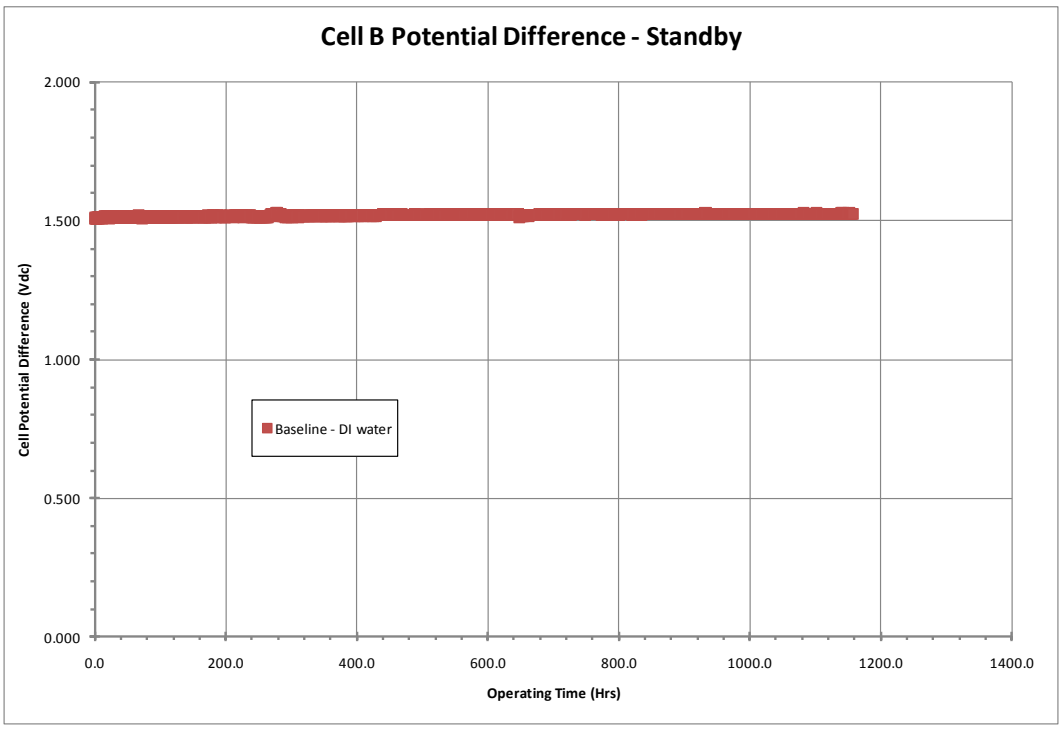

Figure 10. Single Cell Performance - Station B - Standby.

Table 3. Single Cell Performance Data Regression Results Analysis.

SINGLE CELL ID	TEST CONDITION	VOLTAGE DEGRADATION RATE (μV/hr)
Station A (Cell ID 230OGA011-1)	50% production	38
	Standby	9
Station B (Cell ID 230OGA012-1)	50% production	51
	Standby	12

Table 4. Microbiology and TOC Test Results.

Date	Test Description	Microbial Test Results			Comments
		Feed Tank	Loop – Station A	Loop – Station B	
09/11/09	DI baseline	N/A	N/A	N/A	Start of testing
09/14/09	DI baseline	60 CFU/ml	Sample not taken	Sample not taken	Small translucent colonies missed
09/21/09	DI baseline	47 CFU/ml	1420 CFU/ml	213 CFU/ml	Small translucent colonies missed
09/28/09	DI baseline	3.74E+04 CFU/ml	1725 CFU/ml	725 CFU/ml	
10/05/09	DI baseline	5.30E+03 CFU/ml	85 CFU/ml	99 CFU/ml	
10/13/09	DI baseline	1.54E+04 CFU/ml	225 CFU/ml	79 CFU/ml	
10/19/09	DI baseline	1.35E+04 CFU/ml	145 CFU/ml	94 CFU/ml	
10/26/09	DI baseline	7.75E+04 CFU/ml	450 CFU/ml	69 CFU/ml	
10/30/09	DI baseline	Sample not taken	Sample not taken	Sample not taken	End of baseline test

Date	Test Description	Total Organic Carbon (TOC) Test Results			Comments
		Feed Tank	Loop – Station A	Loop – Station B	
09/11/09	DI baseline	N/A	N/A	N/A	Start of testing
09/14/09	DI baseline	0.5 ppm	N/A	N/A	
10/30/09	DI baseline	Sample not taken	Sample not taken	Sample not taken	End of baseline test

N/A = not applicable

VI. Microbial Challenge Test Results

A. 100 CFU/ml Testing

The feed tank was inoculated with the microbial species previously identified on October 30 to provide a concentration of approximately 100 CFU/ml. The initial water sample was taken on November 2 and every week subsequent until the conclusion of the test on December 4. Total test time was 748 hours or approximately 31 days.

Since the 100 CFU/ml inoculum was lower than the baseline concentration, the feed tank remained at greater than 1.0E+03 CFU/ml with a count at the end of the 100 CFU/ml challenge equal to 1.44E+05 CFU/ml. Although baseline microorganisms were the predominant species in the feed tank, the inoculated species including *Cupriavidus metallidurans* and *Ralstonia pickettii* were recovered. The concentrations of bacteria in Loop A fluctuated from 12 to 790 CFU/ml and Loop B ranged from 12 to 250 CFU/ml. During this test period, baseline species from the feed tank were isolated from both Loop A and Loop B. The inoculated *Cupriavidus metallidurans* was isolated from Loop A and *Burkholderia cepacia* was isolated from Loop B.

B. 1,000 CFU/ml Testing

At the conclusion of the 100 CFU/ml test program, the feed tank was recharged with laboratory DI water and inoculated to a target concentration of 1000 CFU/ml on December 4. The first water samples at this concentration level were taken on December 8 and every week thereafter until the conclusion of the test on January 11 when the last water samples were drawn and the tank was recharged with laboratory DI water. Total test time was 823 hours or approximately 34 days.

Bacterial counts in the feed tank following the 1000 CFU/ml inoculation ranged from 1.68E+03 CFU/ml to 1.18E+04 CFU/ml. Genera that were inoculated into the feed tank including *Ralstonia*, *Burkholderia*, *Methylobacterium*, and *Cupriavidus* were now the predominant species. *Caulobacter vibrioides* was not isolated from the feed tank probably due to the fact that it forms stalked cells that adhere to surfaces. Loop A bacterial concentrations ranged from 19 to 98 CFU/ml. Loop B had slightly higher concentrations of bacteria from 32 to 450 CFU/ml. Migration of *Ralstonia*, *Burkholderia*, *Methylobacterium*, and *Cupriavidus* occurred from the feed tank into both loops.

C. 10,000 CFU/ml Testing

The feed tank was re-inoculated on January 13 to bring the microbial population to a target level of 10,000 CFU/ml. The first water samples at this concentration level were drawn on January 18 and every subsequent week until the conclusion of the test on February 15 when the last water samples were drawn and the tank again recharged with laboratory DI water (the sample drawn on January 18 leaked during transport to Boeing, so the first sample analyzed was drawn on January 20). Total test time at this level was 753 hours or approximately 31 days.

After inoculation of the feed tank with the mixed suspension of test microorganisms, the feed tank concentration remained at approximately 5.0E+03 CFU/ml. No significant changes were seen in either Loop A (25 to 59 CFU/ml) or Loop B (28 to 190 CFU/ml). Predominant species in the feed tank and loops continued to include *Ralstonia*, *Burkholderia*, *Methylobacterium*, and *Cupriavidus*. It was hypothesized that the refrigeration of the feed tank were inhibiting the growth of microorganisms greater than 1.0E+04 CFU/ml.

D. DI Water Testing

At the conclusion of testing at the 10,000 CFU/ml level on February 15, the tank was charged with DI water and the single cells continued to operate in a cyclic manner. Refrigeration of the feed tank ceased on March 4 to allow the water in the tank to come to room ambient temperature to permit the microbial population to grow as the attempt to inoculate the tank to 10,000 CFU/ml was unsuccessful (microbial population actually decreased during the conduct of this test objective). Samples for microbial analysis were drawn weekly, with an additional sample drawn on March 16 for complete chemical analysis. Testing with DI water continued until April 15. Total test time with DI water was 1213 hours or approximately 51 days.

Turning off the chiller in the feed tank only resulted in a maximum increase in bacterial concentration to 2.7E+04 CFU/ml. Loop A bacterial concentration increased to 170 CFU/ml and Loop B increased to 260 CFU/ml. *Ralstonia, Burkholderia, Methylobacterium,* and *Cupriavidus* species continued to be present in the feed tank and both loops.

E. 100,000 CFU/ml Testing

The feed tank was inoculated for the final time on April 15 to achieve a target concentration of 100,000 CFU/ml. With refrigeration to the tank still off, the concentration in the feed tank spiked to 1.0E+06 CFU/ml, with both loops registering microbial concentrations between 2100 and 36,000 CFU/ml during the conduct of this test objective. Testing concluded on May 17 with the introduction of fresh DI water into the feed tank; samples were drawn for microbial analysis after charging with DI water, and subsequent samples drawn on May 18 for chemical analysis. Total operating time at these elevated microbial populations was 688 hours or approximately 29 days.

The bacteria concentration in the feed tank was approximately 1.0E+06 CFU/ml following the 100,000 CFU/ml inoculation. *Ralstonia* and *Burkholderia* were the predominant species isolated from the feed tank and likely overgrew the *Methylobacterium*, *Cupriavidus*, and *Caulaobacter*. Also a *Flavobacterium* species was identified in the feed tank at lower concentrations and may be a contaminant. Loop A increased to greater than 1.0E+04 CFU/ml. Predominant species in Loop A were *Ralstonia pickettii* and *Burkholderia cepacia*. *Cupriavidus*, *Methylobacterium*, and *Flavobacterium* were present at lower concentrations. The predominant species in Loop B was *Ralstonia pickettii*. *Burkholderia and Flavobacterium* were also present at lower concentrations. The addition of DI water prior to the end of the test reduced the feed tank concentration to 1.02E+02 CFU/ml. The concentration of bacteria in Loop A dropped to 33 CFU/ml and Loop B decreased to 37 CFU/ml.

VII. Performance Results

The performance curves of cell voltage versus operating time during the microbial challenge test are presented in Fig. 11 through 14. As was the case for the baseline test program, the cell voltage data was parsed according to operating current (50% production versus standby operation) to facilitate analysis of the data using simple linear regression. The results of the regression analyses, presented in Table 5, indicate the performance of both water electrolysis cells was not adversely affected by the addition of the five microbial species identified in the test plan to the feed water up to a maximum feed water tank concentration of over 1.0E+06 CFU/ml. The calculated degradation rates, in microvolts/hour, were in single digits during the conduct of the microbial challenge test and were typically lower than those from the baseline test program.

The results from the microbial and TOC analyses are presented in Tables 6 and 7, respectively. The TOC analysis at the conclusion of the 100 CFU/mL was mistakenly omitted. The concentration of microbial species in the feed tank and recirculating water loops is plotted versus operating time in Fig. 15, with shaded areas of the plot depicting the target concentration level of the feed tank. As can be seen in the plot, the population of microbes in

the feed water tank hovered about 10,000 CFU/mL for most of the test program prior to changing back to DI water regardless of the target concentration, with a spike to 100,000 CFU/mL observed at the conclusion of the 100 CFU/mL level. The concentration saw a steady decline until refrigeration of the feed tank was discontinued; a large jump in concentration to approximately 1.0E+06 CFU/mL was achieved at the end of the program by injecting a high concentration inoculum into the tank. Interestingly the concentration of microbes in the two test loops was essentially two orders of magnitude lower than that in the feed tank, suggesting that the loop environment is not conducive to sustaining growth of the microbial species being tested. This may be attributed to the low pH environment sustained by both loops throughout the duration of the test program.

Both single cells were disassembled on July 20 and the surfaces of different components within the cell itself were swabbed to evaluate microbial activity. Additionally, water samples were drawn from the hydrogen/water phase separators (cylindrical columns manufactured from PFA resin) and the feed water tank for microbial analysis. The results of these analyses are presented in Table 8. Fig. 16 includes a depiction of a single cell assembly and defines the locations of the swabbed areas. A full chemical analysis of the water was also conducted, with the results included in Table 9. This table also includes the results from earlier analyses of water samples drawn from the two water loops (March 16 during testing with DI water at the conclusion of the 10,000 CFU/ml challenge, and again on May 18 at the conclusion of the 100,000 CFU/ml challenge), with a steady decline in pH noted. The relatively low pH of the water in the two test loops as well as the elevated fluoride and sulfate concentrations is indicative of Nafion® degradation due to attack from peroxyl species formed from the catalytic recombination of hydrogen and oxygen diffusing through the polymer membrane. These peroxyl species attack the membrane, releasing hydrofluoric and sulfuric acids as degradation products. A test program with two new liquid-cathode feed single water electrolysis cells is currently underway at HSWL to quantify the fluoride emission rate of the polymer at varying test conditions and its potential impact on the operational performance of the OGA on-board the ISS. The presence of silicon in the water chemistry is consistent with previous analyses of the DI water system at HSWL since silicon as silicic acid is weakly bound to anion exchange resin.

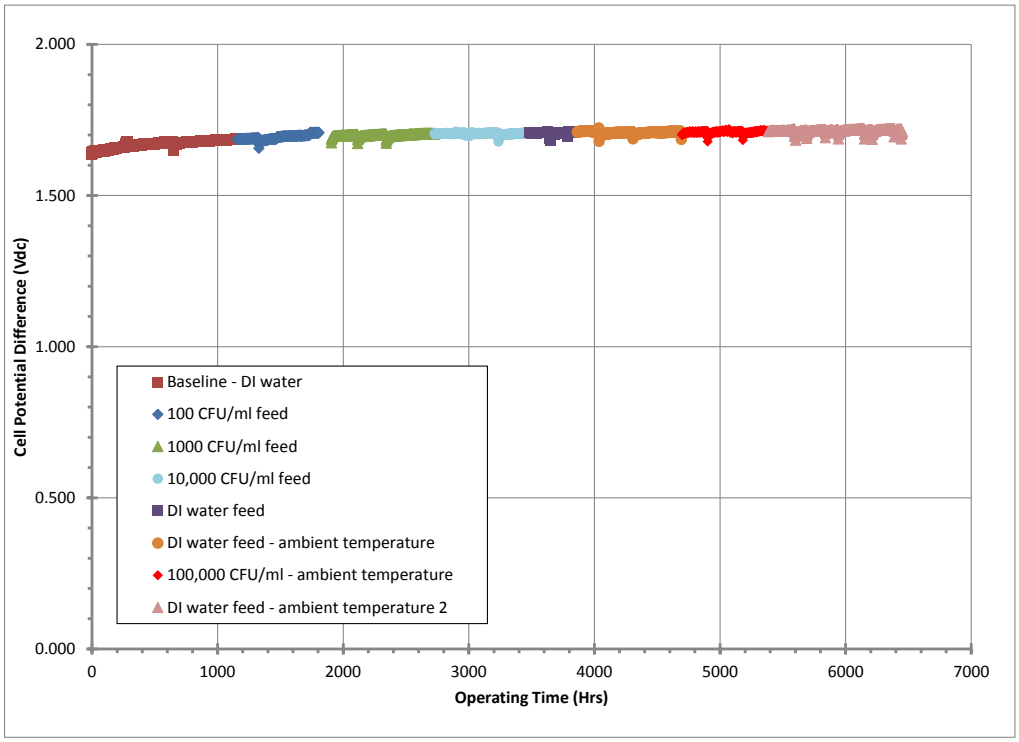

Figure 11. Single Cell Performance - Station A - 50% Production.

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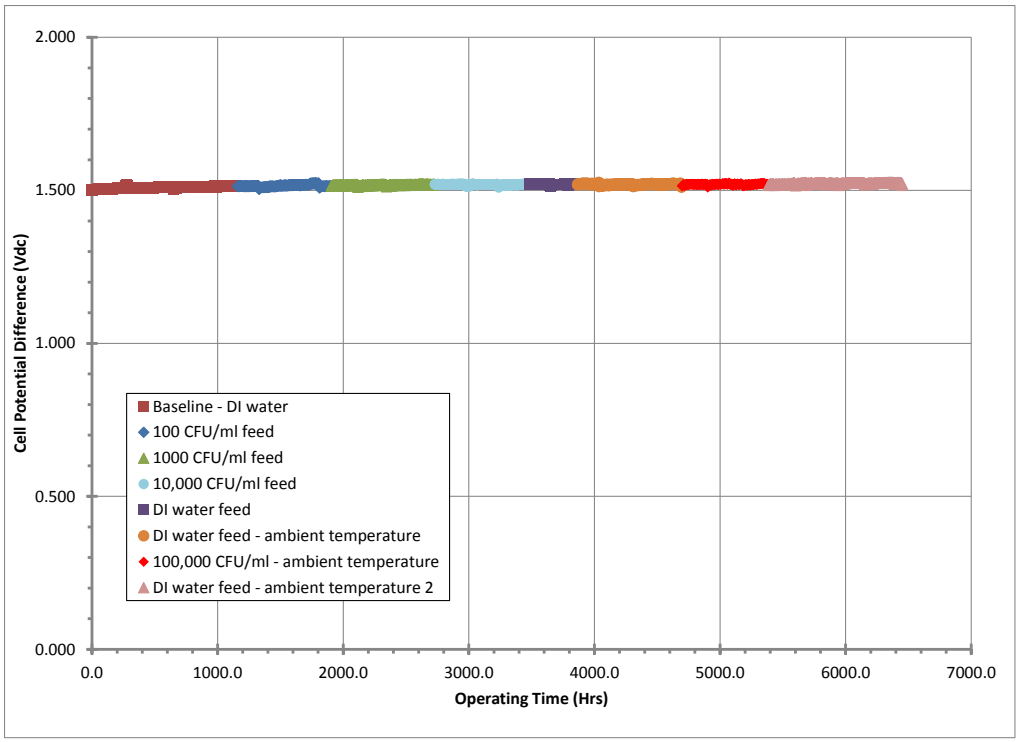

Figure 12. Single Cell Performance - Station A - Standby.

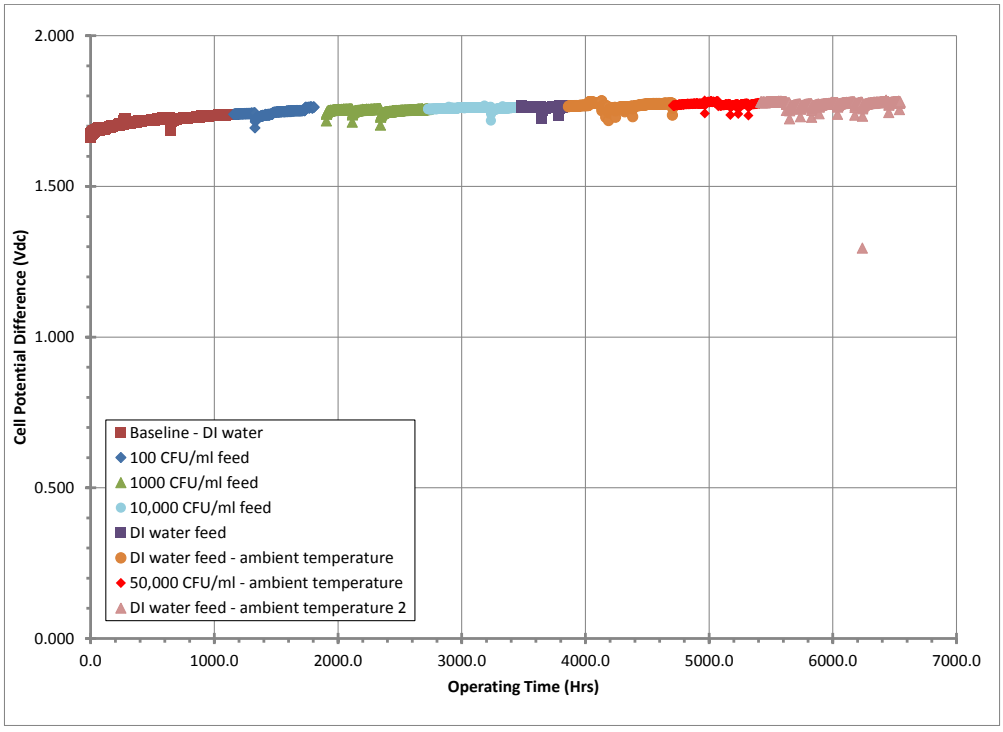

Figure 13. Single Cell Performance - Station B - 50% Production.

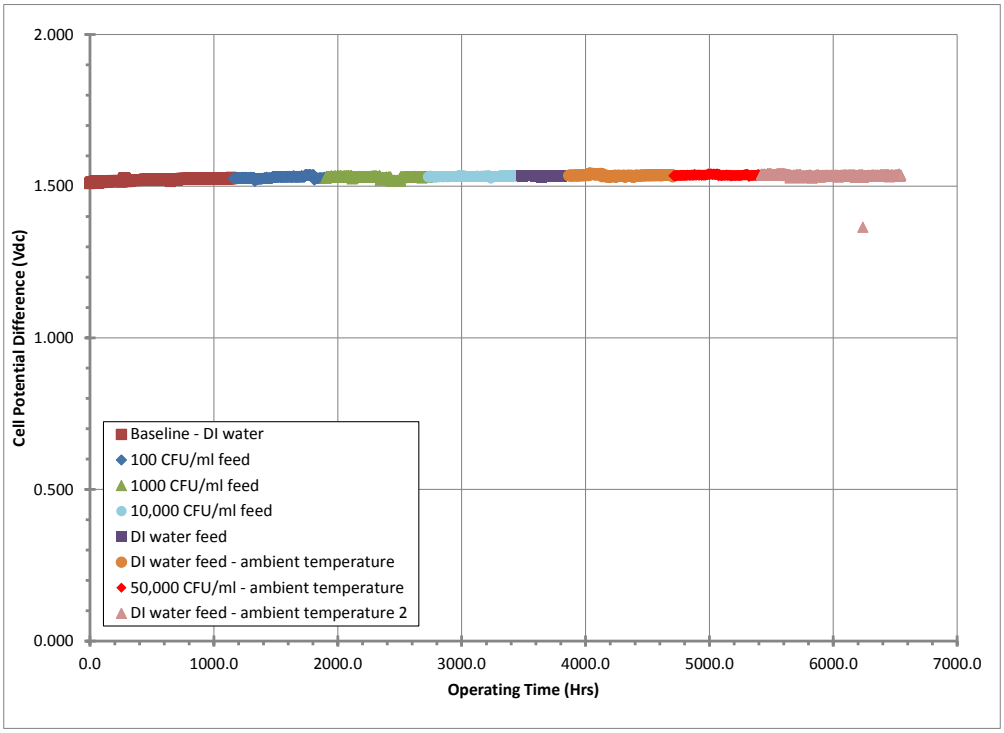

Figure 14. Single Cell Performance - Station B - Standby.

Table 5. Single Cell Performance Data Regression Results Analysis.

SINGLE CELL ID	TEST CONDITION	VOLTAGE DEGRADATION RATE AT VARYING TARGET MICROBIAL POPULATIONS (μV/hr)						
		Baseline DI H_2O	100 CFU/mL	1000 CFU/mL	10,000 CFU/mL	DI H_2O Chilled	DI H_2O Ambient	100,000 CFU/mL
Station A (ID #230OGA011-1)	50% production	38	35	9	-1	9	-2	5
	Standby	9	12	4	-2	0	0	2
Station B (ID #230OGA012-1)	50% production	51	38	2	4	3	6	-1
	Standby	12	12	-6	4	-1	-1	0

Table 6. Microbiology Test Results.

Date	Elapsed Time (Days)	Test Description	Microbial Test Results (CFU/mL)			Comments
			Feed Tank	Loop – Station A	Loop – Station B	
9/11/2009	0	DI baseline	N/A	N/A	N/A	Start of testing
9/14/2009	3	DI baseline	60	Sample not taken	Sample not taken	Small translucent colonies missed
9/21/2009	10	DI baseline	47	1420	213	Small translucent colonies missed
9/28/2009	17	DI baseline	3.74E+04	1725	725	
10/5/2009	24	DI baseline	5.30E+03	85	99	
10/13/2009	32	DI baseline	1.54E+04	225	79	
10/19/2009	38	DI baseline	1.35E+04	145	94	
10/26/2009	45	DI baseline	7.75E+04	450	69	
11/2/2009	52	100 CFU/mL	8.15E+03	290	44	
11/16/2009	66	100 CFU/mL	1.07E+04	39	93	
11/23/2009	73	100 CFU/mL	9.80E+03	46	45	
11/30/2009	80	100 CFU/mL	1.15E+05	790	250	
12/4/2009	84	100 CFU/mL	1.44E+05	12	14	End 100 CFU/mL
12/8/2009	88	1000 CFU/mL	1.68E+03	44	32	Start of testing
12/14/2009	94	1000 CFU/mL	1.18E+04	98	65	
12/21/2009	101	1000 CFU/mL	9.60E+03	48	133	
1/4/2010	115	1000 CFU/mL	5.40E+03	28	77	
1/11/2010	122	1000 CFU/mL	6.10E+03	19	450	End 1000 CFU/mL
1/13/2010	124	10,000 CFU/mL	4.20E+03	25	190	Start of testing
1/20/2010	131	10,000 CFU/mL	5.30E+03	53	48	
1/25/2010	136	10,000 CFU/mL	4.10E+03	29	31	
2/1/2010	143	10,000 CFU/mL	3.00E+03	59	56	
2/8/2010	150	10,000 CFU/mL	4.20E+03	32	28	
2/15/2010	157	10,000 CFU/mL	2.40E+03	38	27	End of test; fill tank with fresh DI water
3/1/2010	171	DI water; chilled	6.60E+02	45	42	Chiller turned off 3/4/10
3/15/2010	185	DI water; amb. temp.	6.10E+03	45	51	
3/22/2010	192	DI water; amb. temp.	8.60E+03	67	55	
3/29/2010	199	DI water; amb. temp.	1.00E+04	147	120	
4/6/2010	207	DI water; amb. temp.	2.70E+04	170	7	
4/15/2010	216	DI water; amb. temp.	1.50E+04	170	260	End of DI water - room; start 100,000 CFU/mL
4/19/2010	220	50,000 CFU/mL; amb. temp.	1.02E+06	3.10E+03	2.10E+03	
4/26/2010	227	50,000 CFU/mL; amb. temp.	6.00E+05	2.20E+04	2.90E+03	
5/3/2010	234	50,000 CFU/mL; amb. temp.	1.06E+06	3.00E+04	1.80E+04	
5/10/2010	241	50,000 CFU/mL; amb. temp.	1.13E+06	2.50E+04	3.60E+04	
5/17/2010	248	DI water; amb. temp.	1.02E+02	33	37	End of 100,000 CFU/mL test; fill tank with DI water and sample. End microbial test program

N/A = not applicable

Table 7. TOC Test Results.

Date	Test Description	Total Organic Carbon (TOC) Test Results			Comments
		Feed Tank	Loop – Station A	Loop – Station B	
9/11/2009	DI baseline	N/A	N/A	N/A	Start of testing
9/14/2009	DI baseline	0.5 ppm	N/A	N/A	
10/30/2009	DI baseline	Sample not taken	Sample not taken	Sample not taken	End of baseline test
10/30/2009	100 CFU/ml	N/A	N/A	N/A	Start of 100 CFU/ml
11/2/2009	100 CFU/ml	0.4	N/A	N/A	
12/4/2009	100 CFU/ml	0.42	Sample not taken(1)	Sample not taken(1)	End of 100 CFU/ml
12/8/2009	1000 CFU/ml	0.31	N/A	N/A	Start of 1000 CFU/ml
1/11/2010	1000 CFU/ml	0.26	0.1	0.13	End of 1000 CFU/ml
1/13/2010	10,000 CFU/ml	0.09	N/A	N/A	Start of 10,000 CFU/ml
2/15/2010	10,000 CFU/ml	0.66	0.36	0.37	End of 10,000 CFU/ml
3/1/2010	DI water - chill	0.23	N/A	N/A	Room temp; 3/4/2010
4/15/2010	DI water - room	0.39	0.25	0.28	End of DI water - room Start 100,000 CFU/ml
4/19/2010	100,000 CFU/ml	0.43	N/A	N/A	100,000 CFU/ml
5/18/2010	100,000 CFU/ml	0.42	0.27	0.20	End microbial test program

N/A = not applicable
(1) Sample mistakenly omitted.

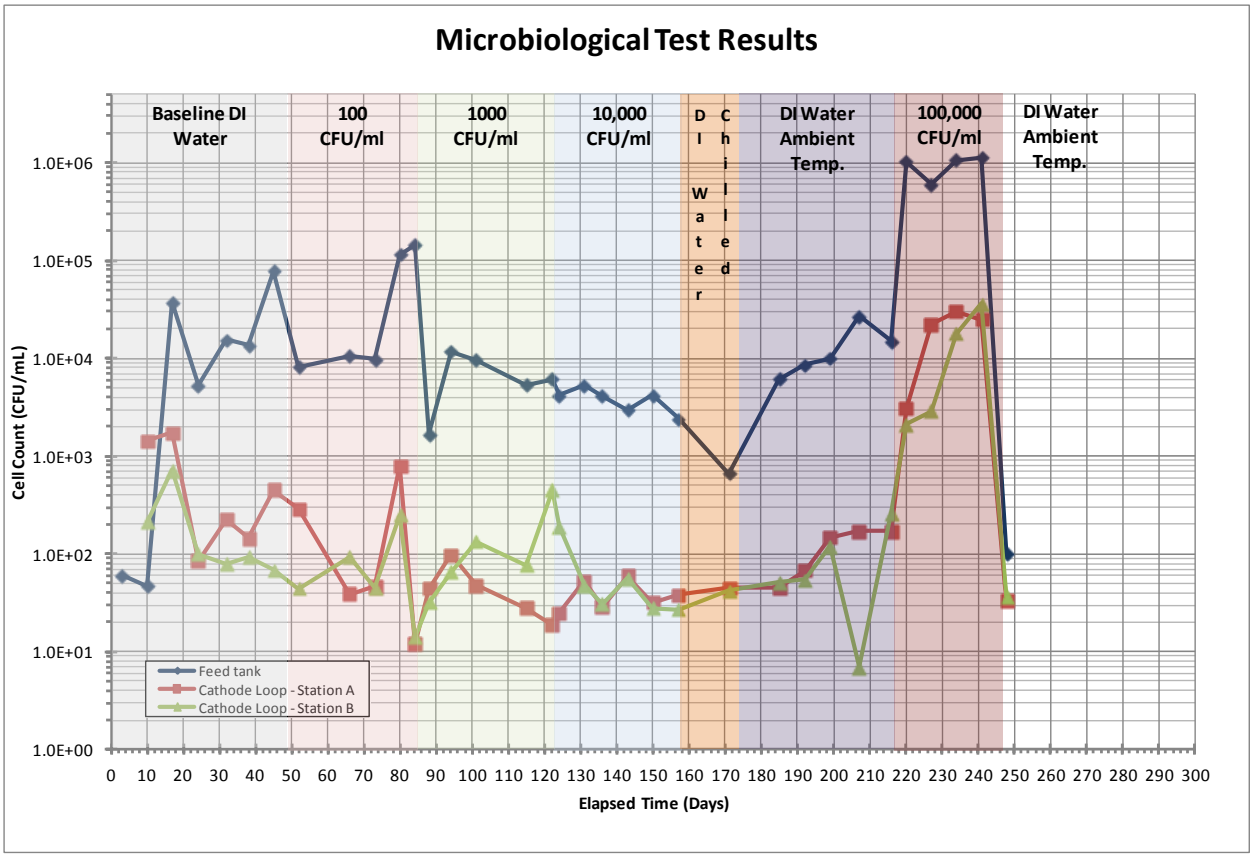

Figure 15. OGA Single Cell Microbiological Test Results.

Table 8. End of Test Enumerations.

Date	Sample Description	Results	Units
7/21/2010	Feed Tank	1.07E+05	CFU/ml
7/21/2010	Recirculation Loop #1	4.50E+02	CFU/ml
7/21/2010	Recirculation Loop #2	9.90E+02	CFU/ml
7/21/2010	Loop A Cathode	8.00E+00	CFU/cm^2
7/21/2010	Loop A Cathode Terminal Separation Sheet	1.40E+03	CFU/cm^2
7/21/2010	Loop A water inlet tube	1.15E+03	CFU/swab
7/21/2010	Loop A water outlet tube	9.50E+02	CFU/swab
7/21/2010	Loop B Cathode	1.60E+02	CFU/cm^2
7/21/2010	Loop B Cathode term separation sheet	1.00E+03	CFU/cm^2
7/21/2010	Loop Bwater inlet tube	1.10E+03	CFU/swab
7/21/2010	Loop B water outlet tube	6.20E+03	CFU/swab

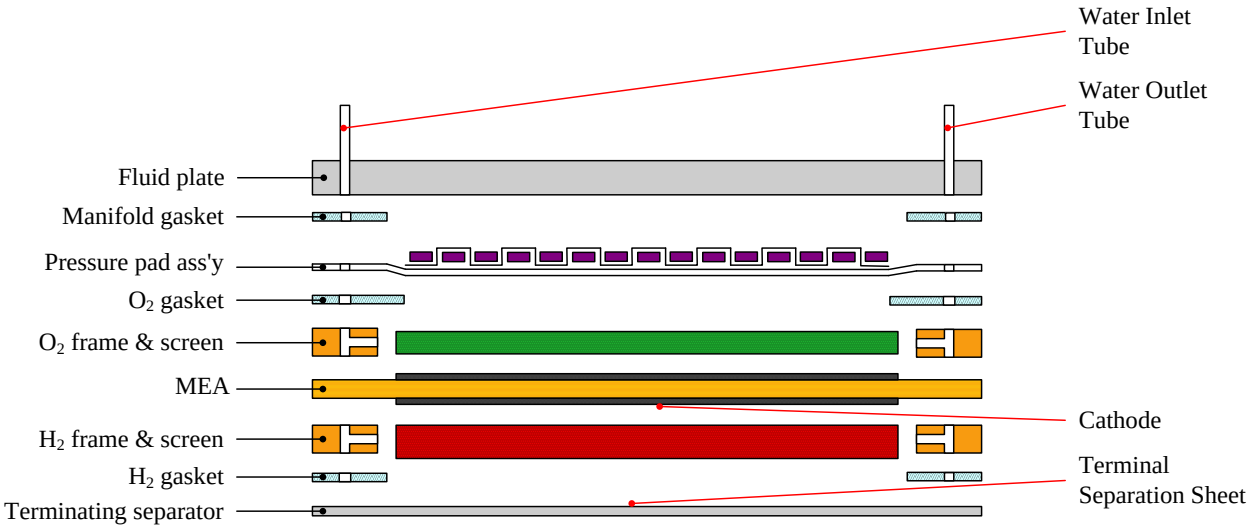

Figure 16. Single Cell Assembly.

Table 9. Water Chemistry Analytical Results.

Parameter / Species	Units	Draw Date						
		3/16/2010	5/18/2010	7/20/2010	3/16/2010	5/18/2010	7/20/2010	7/20/2010
		Loop 1 Results			**Loop 2 Results**			**Feed Tank**
pH	-	4.99	3.98	3.79	4.79	4.04	3.92	6.01
Cond	μS/cm	3.28	13.81	22.70	5.70	21.01	15.74	4.23
TC	ppm	0.21	0.5	0.44	0.32	0.31	0.49	0.51
TOC	ppm	0.17	0.27	0.18	0.13	0.2	0.24	0.51
TIC	ppm	0.04	0.23	0.26	0.19	0.11	0.25	0
F^-	ppm	0.19	1.16	1.27	0.23	0.96	0.92	<0.1
Cl^-	ppm	<0.1	<0.1	<0.1	<0.1	0.10	<0.1	<0.1
NO_3^-	ppm	0.14	0.53	<0.1	0.25	1.22	<0.1	<0.1
PO_4^{-3}	ppm	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
SO_4^{-2}	ppm	<0.1	0.19	0.67	0.33	<0.1	1.21	<0.1
NO_2^-	ppm	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested
Li^+	ppm	Not tested	<0.1	<0.1	Not tested	<0.1	<0.1	<0.1
Na^+	ppm	Not tested	<0.1	<0.1	Not tested	<0.1	<0.1	<0.1
NH_4^+	ppm	Not tested	<0.1	<0.1	Not tested	<0.1	<0.1	<0.1
K^+	ppm	Not tested	<0.1	<0.1	Not tested	<0.1	<0.1	<0.1
Fe Total	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Fe Dissolved	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Cr Total	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Cr Dissolved	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Ni Total	ppm	<0.1	0.11	0.21	<0.1	0.23	0.33	0.19
Ni Dissolved	ppm	0.1	0.11	0.20	0.1	0.23	0.33	0.19
Si Total	ppm	0.7	0.68	1.08	0.85	1.43	1.85	1.06
Si Dissolved	ppm	0.66	0.66	1.09	0.79	1.4	1.88	1.06
Ca Total	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Ca Dissolved	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Mg Total	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Mg Dissolved	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Zr Total	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Zr Dissolved	ppm	<0.1	<0.1	<0.05	<0.1	<0.1	<0.05	<0.05
Mn Total	ppm	Not tested	<0.1	<0.05	Not tested	<0.1	<0.05	<0.05
Mn Dissolved	ppm	Not tested	<0.1	<0.05	Not tested	<0.1	<0.05	<0.05
Pt Total	ppm	Not tested	<0.1	<0.05	Not tested	<0.1	<0.05	<0.05
Pt Dissolved	ppm	Not tested	<0.1	<0.05	Not tested	<0.1	<0.05	<0.05

VIII. Conclusions

The performance of the two single liquid-cathode feed water electrolysis cells was unaffected by the introduction of microbial species in the feed water at the maximum target population of 100,000 CFU/mL. In fact, when the microbial population in the feed water tank spiked to over 1.0E+06 CFU/mL during the conduct of the test the water electrolysis cells continued to operate with no adverse effect on cell performance. Throughout the test program, the microbial population in the two circulating water loops was approximately two orders of magnitude less than the feed tank, indicating the environment in the loop was not conducive to sustaining microbial growth. Degradation products from the degradation of Nafion membrane, specifically hydrofluoric and sulfuric acids, reduced the pH of the recirculating water and may have served to limit the concentration of microbial species within the two test loops. Current testing of two new single cell assemblies is currently underway at HSWL to evaluate the impact these degradation products may have on the operational performance of the OGA on-board the ISS.

This test program evaluated the performance impact of a microbial upset from the potable water bus but did not address the potential impacts of microbial growth and biofilm formation on the membranes during long-term storage of a water electrolysis cell stack as installed in the hydrogen Orbital Replacement Unit (ORU). A program to evaluate disinfecting an ORU for long-term storage to mitigate this occurrence has recently been proposed.

Acknowledgments

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